stability, conformation and binding affinity by equilibrium unfolding using steady state fluorescence and proteolytic digestion assay. These data show that imatinib binds to hFGF-1 and enhances its thermal stability and solvent accessibility. In addition, Biacore analysis was carried out to determine the binding affinity of imatinib to hFGF-1. ^{1}H-^{15}N HSQC NMR was also performed in order to determine exact binding sites and stoichiometry of binding between imatinib and hFGF-1.

273-Pos Board B53
A Shared Binding Site for Propofol and Thiopental in ELIC
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The intravenous general anesthetics propofol and thiopental target pentameric ligand-gated ion channels (pLGIC) and inhibit cation-conducting nAChRs. These drugs also inhibit ELIC, a prokaryotic pLGIC. However, the binding sites for these anesthetics are unknown for either nAChRs or ELIC. Here, using photoaffinity labeling, two-electrode voltage clamp electrophysiology and molecular docking, we identified a functionally relevant binding site for thiopental and propofol in ELIC. Molecular docking identified two binding pockets: an intrasubunit site near M265 of TM3, partially overlapped with the previously identified propofol binding site in GLIC; and an intersubunit site near W220 of TM1, which overlaps with the bromoform binding location identified previously. We generated two mutants, one targeted both predicted binding sites (W220F/W224F/M265C) and another targeted only the intrasubunit site (M265C). Functional measurements on Xenopus oocytes expressing the W220F/W224F/M265C mutant show a significant decrease of anesthetic inhibition, with a five-fold increase in the propofol IC50 and abolishment of thiopental inhibition. Interestingly, the M265C mutation alone could produce the same effect as the W220F/W224F/M265C mutant. Photoaffinity labeling experiments with a light-activated derivative of propofol (aziPm), in conjunction with mass spectrometry, confirmed the binding site at M265 for aziPm. Altogether, the results show that propofol and thiopental bind to a common functionally relevant site. This intrasubunit action site may also be shared by other intravenous anesthetics. Research supported by grants from the NIH.

274-Pos Board B54
Crystal View of Anesthetics and Alcohols Bound in the Pore of ELIC
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Cys-loop receptors, including the acetylcholine, glycine, 5-HT3 and GABA receptors, are molecular targets of general anesthetics and alcohols. Molecular mechanisms of anesthetics and alcohols interacting with Cys-loop receptors are still unclear. ELIC is a prokaryotic homolog of Cys-loop receptors and can be inhibited by general anesthetics and alcohols. Here, we report crystal structures (~3.1 Å) of ELIC bound with the volatile general anesthetic isoflurane, and bound with 2-bromoethanol. The crystal structures were obtained in the presence and absence of the agonist propylamine. Isoflurane was found inside the pore at two sites near T237(6′) and A244(13′), respectively, but 2-bromoethanol was only found near T237(6′). In addition, 2-bromoethanol also bound near Y102 and E150 in the extracellular domain. The presence of propylamine had no obvious effect on the binding sites for both isoflurane and 2-bromoethanol. This is the first time that an anesthetic or alcohol has been observed in the pore at an atomic resolution. The newly identified binding sites of isoflurane and 2-bromoethanol in ELIC are significantly different from previously reported anesthetic and alcohol binding sites. Neither isoflurane binding nor 2-bromoethanol binding introduced significant structural perturbation. The binding of isoflurane and 2-bromoethanol inside the pore suggests the possibility of channel occlusion as a mechanism for channel inhibition of Cys-loop receptors by general anesthetics and alcohols. Supported by grants from NIH.

275-Pos Board B55
Analysis of Antifolate Drugs with Disease Tissue Specificity
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Antifolates, analogues of the essential vitamin folic acid, are used in the clinic to treat cancers and inflammatory diseases. Antifolates are primarily transported into cells via the endogenously expressed reduced folate carrier (RFC). Conversely, our collaborators in Aleem Gangjee's group at Duquesne University have synthesized antifolates (AG antifolates) that transport poorly by the RFC, but are efficiently transported by the folate receptor (hFR). The GPI-anchored hFR is lowly expressed on the apical surface in a subset of normal epithelial lineages, but is highly expressed in many cancers of epithelial origin and on activated macrophages in inflammatory disease. Therefore, AG antifolate molecules have specificity for transport into disease cells over healthy cells. These newly developed AG antifolates cause cell death via inhibition of an enzyme involved in *de novo* purine synthesis, glycinamide ribonucleotide (GAR) transformylase.
We analyzed a series of AG antifolates using biophysical and biochemical techniques to understand both the specificity for transport by the folate receptor as well as the inhibition of the GAR transformylase in order to drive informed, hypothesis-based drug design. Our data, including pH-dependent binding profiles, enzyme inhibition data, and crystallographic models of protein in complex with AG molecules will be presented in the context of drug design and development.

276-Pos Board B56
PKA-Dependent Potentiation Mechanisms of Human CFTR Activity
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Both curcumin and VX-770 potentiate the channel activity of human CFTR (hCFTR) and two most common cystic fibrosis (CF) mutants G551D and F508delta in an ATP-independent but PKA-dependent manner. The underlying molecular mechanisms are unclear. Herein, HEK-293T cells cultured in a Fe^{3+}-containing medium were transiently transfected with hCFTR constructs and curcumin with well-known chemical nature was employed as a template to explore PKA-dependent potentiation mechanisms of hCFTR activity. The results showed that curcumin potentiation of Fe^{3+}-sensitive hCFTR activity was partially weakened by Fe^{3+}-insensitive mutations at the interface of the R domain and intracellular loop (ICL) 3 and completely suppressed by sufficient Fe^{3+}. Thus, release of the inhibitory Fe^{3+}-bound R domain from ICL3 by curcumin may be critical for curcumin potentiation. Further study indicated that curcumin potentiation was significantly prohibited by a missense alanine mutation of F157, Y161 or K166 from ICL1, or R1066, F1074 or F1078 from ICL4, or S795 or S813 from the R domain with or without the involvement of nucleotide-binding domain 2 (NBD2). More importantly, curcumin potentiation was also suppressed by the R811A/S813D or Y808A/S813D mutation and disulfide crosslinking of K162C to S795C enhanced channel opening. Therefore, the phosphorylated R domain may function as a length- and gating-regulatory cross-linker between two transmembrane domains (TMD1 and TMD2). Curcumin may potentiate hCFTR activity by stabilizing the stimulatory ICL1/ICL4-R interactions that promote channel opening by pulling all ICLs together and thus triggering a gating inward-to-outward reorientation of TMDs. Possible chemical interactions may involve cation-π interactions, π-π interactions and hydrogen bonding. Taken together, both release of the R domain from ICL3 and the stimulatory R-ICL1/ICL4 interactions may be necessary for PKA-dependent hCFTR activation and potentiation. These findings may help optimize the potentiators for treating those CF mutants with an ATP-dependent gating defect.

277-Pos Board B57
Kinase Structural Dynamics Enables Tight and Selective Binding of Inhibitors
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Protein kinases are obvious drug targets against cancer due to their central role in cellular regulation. With oncologic diseases being the second leading cause of death in the US kinases rapidly gain attention and are likely to become the number one drug target. Using NMR and fast kinetics, we establish a novel model that solves a longstanding question of high selectivity of clinically relevant drug Gleevec that effectively inhibits Abl tyrosine kinase while closely related Src family of kinases is affected much less. Our study of an entirely different family of Ser/Thr Aurora kinases and its specific inhibitors suggests that an energy landscape that provides tight affinity via an induced-fit and binding plasticity via a conformational selection mechanism is likely to be general for many inhibitors.

278-Pos Board B58
Glutathione Reductase of *Plasmodium Falciparum* as an Antimalarial Drug Target of Methylene Blue
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Plasmodium falciparum is the cause of human malaria and is one of two malaria parasites known to have drug resistance. Since there are no preventative

vaccinations against malaria, the control of this disease is heavily dependent upon the use of antimalarial drugs. Antimalarial drugs, such as methylene blue, are effective therapies against human malaria. At a specific concentration, methylene blue has been shown to be a selective inhibitor of the parasite's glutathione reductase (*Pf*GR).
Glutathione reductase is an important target when studying malaria drug resistance because it is a flavoenzyme that regenerates glutathione, which is an essential protein for antioxidant defense against cell damage. Methylene blue is also a substrate that is reduced by gluthathione reductase to produce leucoMB. This is then spontaneously oxidized by molecular oxygen to form methylene blue again. During this process, reactive oxygen species, such as hydrogen peroxide and superoxide form. These act as recycling catalysts against infectious organisms. Due to *Pf*GR's central position in redox control, it is ranked number one as an antimalarial drug target. The goal of this research is to study the interface between methylene blue and the putative protein target, glutathione reductase, in order to understand the drug action mechanism. *Pf*GR was expressed and purified and hydrogen-deuterium exchange (HDX) will be used to map the drug-protein interface. We will present the mass spectrometry data for the solvent exposed peptides after digestion with pepsin when *Pf*GR is complexed with methylene blue, which will allow us to narrow down the active site.

279-Pos Board B59
Effects of Molecular Crowding on the Binding Affinity of Dihydrofolate for Dihydrofolate Reductase
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The reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (DHFR), using NADPH as a cofactor, is an essential part of the folate cycle. The inhibition of DHFR leads to interruption of DNA synthesis and consequently cell death, making this enzyme a crucial target in the treatment of cancer and other diseases. Previous studies examined the effects of small molecule osmolytes on the substrate interactions with two non-homologous DHFRs, E. coli chromosomal DHFR (EcDHFR) and R67 DHFR, with vastly different active site structures. The results indicated that DHF weakly interacts with the osmolytes in solution, shifting the binding equilibrium from DHF bound to DHFR to unbound DHF. It is hypothesized that similar weak, nonspecific interactions may also occur between cellular proteins and DHF. Weak interactions between cellular proteins and DHF would have consequences in vivo, where the concentration of the cellular milieu is approximately 300 g/L. Under the crowded conditions in the cell, there is a higher propensity for intermolecular interaction.
Crowding effects of macromolecules in concentrations similar to those in vivo were examined. Isothermal titration calorimetry (ITC) and enzyme kinetic assays were used to detect effects of molecular crowders by monitoring activity of the (DHFR)-NADPH or DHF complex and the ternary DHFR-DHF-NADPH complex in the presence of these crowders. To recreate the conditions of molecular crowding in vivo, the binding of the enzyme-ligand complexes in the presence of molecular weight crowding agents (lysozyme or casein) was examined. Analysis of the Kd's and Km's indicated a correlation between increased molecular crowding in the solution and weakened binding of the DHFR-substrate complexes. These findings indicate an importance of molecular crowding on EcDHFR activity in vivo.

280-Pos Board B60
Quantifying the Influence of the Crowded Cytoplasm on Small Biomolecule Diffusion via Homogenization Theory
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Cytosolic crowding is known to influence the thermodynamics and kinetics of in vivo chemical reactions. Crowders, including proteins, macromolecular assemblies and intracellular organelles, reduce the volume available to a diffusing substrate and thereby lower its effective diffusion constant relative to its rate in bulk solution. However, the nature of a substrate's interaction with crowders, such as through electrostatic or van der Waals forces, can further influence the effective diffusion rate. To probe the impact of crowding over micron-scale intracellular distances, we apply a multi-scale mathematical theory, homogenization, to estimate effective diffusion rates for ions and small biomolecules diffusing in a densely-packed lattice of representative cytosolic proteins. Specifically, via the finite element method we numerically solve the homogenized diffusion equation for a nearly 1 micron cubed cytosolic fraction based on published Brownian dynamics data of the bacterial cytoplasm (McGuffee and Elcock, PLOS Computational Biology, vol. 6, no. 3, p. e1000694, Mar. 2010). Our simulations quantify how the crowded volume fraction, irregularity of protein shapes and distribution, and molecular interactions influence the diffusion rates of small molecules.

281-Pos Board B61
Specific or General - It is All About Solute Interactions with the Pore
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Recently, Kojima and Nikaido (PNAS, 110: E2629, 2014) examined the idea of potential specificity of porin channels, where -lactam antibiotics' interaction with the pore-lining residues, demonstrated *in vitro*, was suggested to facilitate transmembrane transport (PNAS, 99:9789, 2002). The authors came to a conclusion that the binding inside OmpF channel does not significantly affect the penetration of ampicillin and benzylpenicillin.
While we emphatically agree with the authors that attractive interactions *per se* do not constitute the leading imperative in search for an antibiotic with the ''magic bullet'' potential, it would be surprising if Nature (or pharmaceutical companies, by trial and error) had not explored the benefits of these interactions to facilitate antibiotic translocation. Indeed, the probability of translocation through the OmpF pore for the molecule of ampicillin size that is already at the channel entrance could be estimated as a fraction of one percent (JCP, 116:9952, 2002). This is an impressively small number.
The presence of optimal attractive interactions is able to compensate for the entropic cost of confinement (Biochemistry, 52:9246, 2013) and thus increase the translocation probability to 0.5, its maximum value for passive, although interaction-assisted, diffusion. Certainly, not every attractive interaction is optimal or even beneficial for translocation. Too strong or wrongly distributed binding can be detrimental. However, attractive interactions in a particular channel-solute pair are able, at least in principle, to transform a porin, which is ''general'' for many other solutes, into a ''specific'' one for the particular pair.

282-Pos Board B62
Using Sedimentation Velocity to Investigate the Nucleotide-Linked Assembly of E. coli ClpA
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The AAA+ (ATPases Associated with various cellular Activities) group of proteins is a large superfamily whose members are present in single-celled and multi-celled organisms. This family is subdivided into two classes; Class I ATPases contain two ATPase domains per monomer while Class II ATPases contain one ATPase domain per monomer. Both classes utilize ATP binding and hydrolysis to undergo conformational changes that allow them to perform mechanical work. Further, the biologically active form for most members of the AAA+ family is a hexamer. Quantitatively examining the biologically-relevant activities for AAA+ proteins requires a precise determination of the concentration of hexamers in solution. The difficulty in determining the hexamer concentration is that the oligomeric state of interest often resides in a dynamic equilibrium. This leads to a distribution of oligomeric states in solution. Therefore, insight into nucleotide-linked assembly is required to be able to understand how any of the AAA+ motors function. ClpA is a Class I AAA+ protein. Apparent self-association equilibrium constants were obtained using sedimentation velocity and were subsequently analyzed using binding polynomials that incorporate either zero cooperativity or infinite cooperativity into the nucleotide binding process to yield apparent nucleotide binding constants. Here we show that the population of ClpA dimers, tetramers, and hexamers is dependent upon nucleotide concentration. Further, apparent nucleotide binding constants were found for each oligomeric state. The above-stated information was used to generate a prediction of ClpA hexamer population that takes into account nucleotide-linked assembly. We anticipate the results of this study will set the stage for being able to predict the concentration of hexamers at any given nucleotide concentration, therefore allowing us to probe the kinetic and energetic properties of ClpA as well as other members of the AAA+ superfamily.

283-Pos Board B63
Macromolecular Crowder and Ligand Compete for the Closed Domain Cleft of Maltose Binding Protein
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Protein-ligand binding is central to many biochemical processes including enzyme catalysis/inhibition and cellular signaling. In the cellular context, these processes occur in milieus crowded with bystander macromolecules. Growing evidence suggests that the macromolecular crowders are not inert but influence the biochemical processes. A previous study of our lab demonstrated that a synthetic polymer crowder, Ficoll70, and a ligand, maltose, compete for binding with the maltose binding protein (MBP), a periplasmic protein involved in nutrient uptake and chemotaxis [Miklos and Zhou, PLoS ONE 8, e74969 (2013)]. Fluorescence and NMR spectroscopy showed that Ficoll70 weakly binds to MBP and this